REMARKS

Please consider this paper as a petition for a 3-month extension of time. Please charge any required fees to have this petition and response entered to our deposit account No. 500687.

Reconsideration and allowance are respectfully requested.

Claims 2-7, 11-14, 31-36, 39, 40, 42-60, and 62-66 are pending in the application.

The rejection of claims 34, 35, 41, 45, 61, 62 and 65 under 35 U.S.C. § 112, first paragraph, is obviated in part and respectfully traversed in part. The part of the rejection regarding claims 34, 41, 62 and 65 is obviated by the amendments to the claims set forth above. Claims 41 and 65 have been cancelled without prejudice and Applicant reserves the right to pursue the subject matter of these claims. Claims 34 and 61 have been amended to recite the specific language used in the examples disclosed in the originally filed application, as suggested by the Examiner. As described in the originally filed examples, "the enzyme activity of the diluted enzyme solution is at least statistically equivalent to the enzyme activity of the enzyme solution before dilution." No new matter has been added.

Claims 35, 45 and 62 recited that the solution is enhanced by at least 200%. Page 4, lines 10-16 of the original specification teaches that (1) the diluted enzyme has at least the same level of activity as the undiluted enzyme and (2) a minimum dilution factor of 3 times. A dilution factor of 1 is no dilution (0%), a dilution factor of 2 is doubling (100%) and a dilution factor of 3 is tripling (i.e. 200%). The dilution factor of 3 (200%) is the minimum, i.e. at least 200%. Thus, if a 200% dilution has the same level of activity as the undiluted enzyme after treatment according to the present invention, then "the activity of the enzyme solution is enhanced by at least 200%." The original application provides clear support for claims 35, 45 and 62.

Furthermore, Example 1 teaches an increase in activity from 2035 U/mL, the

activity of the raw enzyme solution before subjecting said enzyme to the present invention, to about 11000 U/mL, or about 5.4 times the activity of the original amylase formulation. In percentage terms, if the observed enzyme activity per mL of raw enzyme had been 2035 U/mL, it would be a 0% enhancement. If activity had increased to 4070 U/mL, it would be a 100% enhancement. If activity had increased to 8140 U/mL, it would be a 300% enhancement. As stated in example 1, the activity per mL of raw enzyme, increased to 11000 U/mL. Therefore, in percentage terms, activity per mL of raw enzyme, increased by 440%, or 5.4 times. Therefore, Examples 1-3 provide further support for enhancing enzyme activity by at least 200%. No new matter has been added.

Applicants submit that the claims fully comply with Section 112. Accordingly, withdrawal of the Section 112, first paragraph, rejection is respectfully requested.

The rejection of claim 66 under 35 U.S.C. § 112, second paragraph, is obviated by the amendment to claim 66 set forth above. Accordingly, withdrawal of the Section 112 rejection is respectfully requested.

The rejection of claims 2-7, 11-14, 31, 34, 42 and 46-65 under 35 U.S.C. § 112, second paragraph, is respectfully traversed in part and is obviated in part. The part of the rejection regarding claims 34 and 61 is obviated by the amendments to the claims set forth above.

On page 3 of the Office Action, the Examiner argues that:

> It is unclear what is meant by "raw enzyme and raw enzyme weight." Although, applicant defines "raw enzyme and raw enzyme weight" it is suggested that "crude enzyme" may better describe applicant's invention. Applicant states that "raw enzyme solution" is defined by their specification, which this is true, applicant refers to the raw enzyme solution as the crude enzyme solution in the specification. Further, applicant fails to present "enzyme weight" in a standard form of activity as is known in the art and as recommended by Methods of Enzymology. A clarification with respect to applicant's enzyme method is advised.

As admitted by the Examiner, the term "raw enzyme" is clearly defined on page 2, lines 26-36 of the originally filed specification as follows:

> By the term "raw enzyme solution" in this specification is meant a commercial grade formulation, produced by fermentation from any one of a variety of bacterial and microbial sources. In the case of an extracellular enzyme, the crude enzyme extract is obtained by, e.g., filtration or centrifugation of the fermentation broth, thus isolating the enzyme from protein debris. If the enzyme is produced intracellularly, the cells are lysed prior to filtration/centrifugation. The crude enzyme extract may also be subjected to membrane separation, ion exchange, or ultrafiltration to produce a partially purified, concentrated enzyme extract rich in the desired enzyme, and relatively devoid of other competing/contaminating enzymes and/or cells. The enzyme solution may also include residual components from the fermentation medium, protease inhibitors, and stabilizing agents.

Thus, the term "raw enzyme" clearly complies with Section 112. The term "enzyme weight" is clearly defined on page 3, lines 23-30 of the originally filed specification as follows:

> By the term "raw enzyme in connection with its weight" as used in this specification and claims is meant the volume of the raw enzyme solution x the density of the raw enzyme solution.
>
> The weight ratio of raw enzyme to purifying agent is dependent on the enzyme and purifying agent. Preferably, the ratio is not greater than 50:1, more preferably, not greater than 25:1, and still more preferably not greater than 15:1. A preferred ratio for use with activated carbon as the purifying agent provides 11g raw enzyme purified with 0.75 g activated carbon.

Thus, the claimed phrase "raw enzyme weight to activated carbon weight ratio" is clearly defined in the specification and fully complies with Section 112. Accordingly, withdrawal of the Section 112 rejection is respectfully requested.

Response to Prior Art Rejections:

The rejection of claims 1-6, 9, 10, 31-33, 39, 40, 42-44, 46-52, and 63-66 under 35 U.S.C. § 103(a) as being unpatentable over Lausten in view of Schuster is respectfully traversed. The rejection of claims 2-6, 12, 13, 31-33, 39, 40, 42-44, 46-61 and 63-66 under 35 U.S.C. § 103(a) as being unpatentable over Lausten in view of Schuster and Shenoy is respectfully traversed. The claimed invention is not taught or

suggested over the theoretical combination of references for the many reasons of record and for the following reasons. Applicant responds below to every new argument raised by the Examiner.

Laustsen is the primary reference cited by the Examiner. Laustsen teaches a purification method that results in a 12% increase in flux. See page 2 of Laustsen's 2 December 2002 Response, submitted previously. Laustsen does not provide any disclosure or even a suggestion of how to improve enzyme activity so that less amounts of enzyme are necessary.

Schuster also does not observe the effect of activated carbon on the activity of the purified enzyme solution. In fact, a necessary condition of Schuster's observations is the presence of tightly bound ligands such that the treatment with activated carbon can remove these tightly bound ligands allowing the enzyme to attack the substrate uninhibited. Since there are very few, if any, inhibitors in the 'raw enzyme solution', which is a partially purified enzyme solution, one skilled in the art would not anticipate that the activated carbon would have any effect. While Schuster treats purified P450 with activated carbon, there is no attempt to quantify any increase in activity of the enzyme after such treatment. Schuster merely tests the ability of the activated carbon to release an antibiotic that has been added to the purified protein *in vitro*. This is very different from the present invention where a partially purified raw enzyme solution has been filtered and clarified (page 2 lines 29 to 34) to obtain an enzyme solution devoid of endogenous inhibitors. In light of Schuster, one skilled in the art would believe that, in the absence of any inhibitors or other tightly bound ligands, activated carbon would have no positive effect. Thus, the combination of Lausten and Schuster cannot teach or suggest the claimed method of enhancing enzyme activity.

Shenoy also does not provide the deficiencies of Lausten and Schuster. Shenoy discusses the purification and properties of glucoamylases from different fungal sources, using circular dichroism (CD) to examine effects of pH, T, substrate and denaturants. The binding kinetics and reaction mechanism are also discussed.

Shenoy notes that near-UV CD at optimal pH depends on the fungal source. A.

niger and A. candidus GA showed extra peaks at 272-275 nm; troughs observed with A. niger and A. candidus were not observed with Rhizopus species. Glucoamylases from different sources had different aromatic amino acids and cysteine content. The bottom line is that the species used to produce the glucoamylase affects the near-UV CD spectrum. The far-UV spectra also depend on species. Slight differences in magnitude and band position were observed in the 208-210nm and 217-220nm range. Some of the subtle shifts in band position are thought to be due to carbohydrate moeities in the enzyme. Enzymes from different species contain the same amount of alpha helix, but differ in beta-sheet structure. Shenoy teaches that pH affects secondary structure and activity of glucoamylase. Maximum activity occurred at pH 4.8, which corresponded to the highest percentage of alpha-helix and lowest percentage of beta-sheet in all three species - A. niger, A. candidus, and Rhizopus. The lowest activity (pH 10) corresponded to higher levels of either beta-sheet or gamma (disordered structure) content. A higher pH causes ionization of tyrosine, leading to unfolding and loss of activity, consistent with involvement of ionic linkages in the native conformation. Shenoy also shows that increased temperature reduces helical content, ultimately leading to unfolding at higher temperatures (> 60ºC). Exposure to urea reduced alpha-helix content; this paralleled a decrease in activity. Addition of substrate also reduced the alpha-helix content, while increasing the beta-sheet content. However, near-UV bands were unaffected. Carbohydrate groups were necessary for stability, but not for activity. Perhaps the CHO moieties stabilize the enzyme against heat denaturation. Reduction of the enzyme (with and without periodate treatment) confirmed importance of the CHO for structural stability.

Essentially, Shenoy has used CD to show structural differences between different sources of glucoamylase, and structural changes that occur as activity changes. In other words, following a change in activity due to a change in pH, temperature, or chemical treatment, CD can be used to elucidate the structural changes that result from such a treatment or process. One cannot, however, use a change in CD spectrum to develop a process, or to predict that a particular treatment would lead

to a specific change in activity. Indeed, as noted by Alliance Protein Laboratories on their website, even though one can use CD to establish the approximate percentage of alpha-helix content, "it cannot determine which specific residues are involved in the alpha-helical portion". Applicants have also shown that the alpha-helix content of different amylases is highly dependent upon the source enzyme (Figure 3 of instant specification), but all of these enzymes are highly active, so the CD spectrum cannot be a unique predictor of activity. Thus, even though the secondary and tertiary structures of these enzymes are different, they are all active. Therefore, a specific secondary structure or a specific tertiary structure is not essential for activity, but a range of such structures may produce an "active" conformation. Shenoy simply does not teach any methods for increasing enzyme activity. Thus, the combination of Lausten, Schuster and Shenoy cannot teach or suggest the claimed method of enhancing enzyme activity. For this reason alone, the Section 103 rejection should be withdrawn.

Even if the cited references were combined, the experimental evidence of record rebuts any prima facie case of obviousness based on Laustsen, or any other reference that only teaches purification. The experimental evidence now of record demonstrates that the claimed invention results in a surprising 200 to over 900% increase in enzyme activity.

Within the originally filed patent application, the April 26, 2007 rule 132 declaration, the 13 March 2008 rule 132 declaration, and the numerous articles of record, Applicants have conclusively demonstrated that:

1) Merely contacting undiluted commercial enzyme through activated carbon provides no measurable increase in enzyme activity.

2) Raw fermentation broth containing cells diluted by ~55% (approximately 1:1, the maximum taught by Laustsen) cannot be processed through activated carbon by the claimed method. The negligible permeability is due to the presence of cells in

the system, Applicants' use of high levels of activated carbon, and the fact that Applicants' process can be practiced under ambient pressure.

3) Raw fermentation broth which has cells removed and has been substantially diluted, e.g., 10-fold, or 1 part broth with 9 parts water/buffer, *can* be processed through the large quantity of activated carbon specified in the present patent application.

4) Commercial enzyme that has simply been diluted is well known in the art to have reduced activity, which is further confirmed by the experimental results in the rule 132 declarations. In contrast, processing the diluted enzyme through activated carbon (as specified in the present patent application) leads to an unexpected significant (multi-fold) enhancement of activity.

5) Purification, as understood by those skilled in the art, does NOT automatically lead to an increase in enzyme activity. As demonstrated by the numerous references of record, a reduction in activity after purification is far more likely. Thus, purification methodologies are designed to minimize this well known adverse outcome

6) Purification, as understood by those skilled in the art, may lead to an undesirable change in structure and, thus, a change in CD or UV spectrum. This change in structure (UV and CD spectrum), as outlined in point (5), is known to be deleterious, and causes a REDUCTION in enzyme activity. Thus, conventional purification methodologies use structural analysis post-purification to ensure that structure has been PRESERVED, since this corresponds to retention of activity or minimizes the reduction of activity.

7) In an embodiment of the present invention, the interaction between the diluted purified enzyme and the activated carbon leads to a desirable structural change in the protein, as evidenced by a change in CD/UV spectrum and electrophoretic bands that is surprisingly not deleterious. Unlike conventional purification, this change in structure provides a heretofore unknown multi-fold INCREASE in activity. It is Applicants' hypothesis, without being bound by any theory, that this unexpected and substantial increase in activity arises from activated carbon's role as a catalyst and Applicants therefore refer to a catalytic or chemical change in protein structure, the result of which is an increase in activity. While activated carbon is known to have catalytic activity, it was unknown prior to the present invention that activated carbon could catalytically increase the activity of a purified and diluted enzyme, and surely not to such a large degree, up to 1,000%.

The present invention is distinct from Laustsen because the present invention requires the combination of: (a) removal of cells (purification) and Laustsen does not, (b) much higher levels of dilution (at least three-fold, and preferably 5-fold or 10-fold, while Laustsen cites, at most, a 1.1-fold dilution, to 45% of the original protein concentration), and (c) a high ratio of activated carbon to enzyme to provide the claimed enhancement of enzyme activity.

Applicants note that Laustsen discloses in paragraph 46 that "[a]ccording to the present invention the added amount of carbon is preferably from 0.05 to 2% (w/w) of the initial fermentation broth volume, in particular the added amount of carbon is from 0.1 to 1% (w/w) of the initial fermentation broth volume." At the maximum of 2%, this is a 50:1 range of fermentation broth to activated carbon. However, Laustsen clearly teaches to use amounts of activated carbon in a direction away from the claimed invention, i.e. far less activated carbon. First, Laustsen's preferred range is 0.1 to 1%, which at 1% is a 100:1 ratio. Second, all of Laustsen's working Examples 1-3 teach far less activated carbon, i.e., 0.2%, which is a 500:1 ratio. Furthermore, the attached Rule

132 Declaration demonstrates that even at low activated carbon amounts, 500:1, the process of Laustsen did not work unless pressure was applied and, thus, the much higher amounts of carbon claimed (not greater than 50:1) would cause further problems in the process of Laustsen, as discussed in the Rule 132 Declarations and below. In contrast, in the claimed invention the ratio of broth to carbon is not greater than 50:1. Note that a ratio of 49:1 has more activated carbon than a ratio of 50:1. Applicants further point out that the claimed activated carbon ratios are used in combination with other claimed steps, such as at least a 3 fold dilution and filtering the cells before contacting with activated carbon for a time sufficient to effect enzyme enhancement, which combination is not taught by Laustsen.

All of the prior art rejections rely upon Laustsen (US2002/0020668). Applicants submitted two Rule 132 Declarations that clearly demonstrates the differences between Laustsen and the claimed invention.

The starting materials used in Example 2 of Laustsen are not all commercially available. Thus, Applicants contracted a fermentation facility to produce Laustsens' alpha amylase in accordance with the British patent 1,296,839 cited by Laustsen,, and then attempted to process the raw fermentation broth according to the conditions and ratios described by Laustsen in Example 2. For comparison, Applicants then also performed experiments using the fermentation broth diluted to 10% of its original concentration (a dilution ratio according to the present invention), and Applicants also performed experiments with an essentially cell-free enzyme preparation, following the claimed invention. Experiments were performed with the low level of activated carbon specified by Laustsen, and with the high level of activated carbon specified in claimed invention. Details of the experiments and the experimental observations are described in the Rule 132 Declarations.

Applicants original plan was to test the activity of the enzyme processes according to the procedure described by Laustsen. However, when Applicants ran the process according to Laustsen, none of the enzyme eluted, even though a more porous filter was used, so Applicants did not have a sample to test. Increasing the amount of

activated carbon, as per the present invention, did not improve the situation. Applicants were only able to collect samples from trials where the conditions were dramatically modified from those described by Laustsen, e.g., (1) by using much more diluted fermentation broth (1 part broth in 9 parts water, versus 1 part broth plus ~1 part water, as per Laustsen), (2) removing the cells first, as per the present invention, or (3) using a highly porous filter cloth. Even in the case of method (3), once the cells accumulated on the surface, Applicants could not collect much enzyme from the broth. Ultimately, these experiments confirmed that the claimed process is dramatically different from that described by Laustsen, and that cell removal and significant pre-dilution are key prerequisites to using the claimed process.

Furthermore, Applicants have demonstrated that large quantities of activated carbon are a disadvantage when the goal is filtration (Laustsen), but Applicants own work has also shown that large quantities of activated carbon are essential if the goal is activity enhancement. This further distinguishes the claimed method from Laustsen's. Since Laustsen's stated goal is filtration improvement, Laustsen cannot teach the use of large quantities of carbon, because it adversely affects filtration rate (flux). Without sufficient carbon (or retention time in the carbon), it is difficult to detect any change in enzyme activity if any actually occurs. Conversely, the present invention must use high levels of activated carbon, otherwise, Applicants do not get the claimed enhancement of enzyme activity. Too little activated carbon is a disadvantage for the present invention, whereas too much activated carbon is a disadvantage for Laustsen and, thus, Laustsen teaches in a direction away from the claimed invention. Given the claimed invention's distinct objectives, the operating conditions, dilution levels, and carbon loading must be distinctly different from the process of Laustsen.

Laustsen is owned by Novozymes, which is one of largest producers of enzymes in the world. Thus, Novozymes is highly skilled in the art of producing enzymes. From an economic perspective, given the significant shortage of fermentation capacity in the U.S., and the high cost to produce and ship enzymes, it would be enormously surprising if Novozymes failed to adopt the presently claimed technology if they found an activity

enhancement from their work with the Laustsen application. The present invention conservatively reduces enzyme costs by 10 to over 90% in a multi-billion dollar industry. Certainly, Laustsen, as a Novozymes employee, would therefore have primarily emphasized and claimed any results of enhanced activity, rather than results of enhanced permeation, if enhanced activity had been observed. Instead, the obvious conclusion is that enhanced activity was neither detected, nor expected by Laustsen. As noted above, to achieve their goal of enhanced microfiltration, Laustsen has to use small amounts of activated carbon to avoid clogging the filter, insufficient to create the activity enhancement seen (or possible) with the activated carbon loadings used in the presently claimed process. Thus, Laustsen actually teaches in a direction away from the claimed invention.

More specifically, the Rule 132 Declarations confirm that:

(1) A raw fermentation broth, even when diluted by ~55% as specified by Laustsen, cannot flow through the column. Even with very low quantities of activated carbon (i.e., 0.3g per 310 mL of diluted fermentation broth (1050:1 ratio)), there is no permeation through the column and column frit even though the frit utilized had a pore size of 20µm, which was far larger than Laustsen's disclosed pore size of 0.45µm. Thus, Laustsen's microfiltration process is dramatically different from the claimed method, which uses the combination of contact with high levels of activated carbon for a time sufficient to effect enzyme enhancement, much higher dilutions, and an essentially cell-free solution. Furthermore, Laustsen applies pressure across the membrane in order to achieve the stated fluxes, whereas the claimed invention is not limited to any particular pressure.

(2) If the porosity restriction imposed by the column frit is removed in Laustsen, e.g., by replacing the column frit with filter cloth, some very limited permeation of the diluted fermentation broth is observed, but the rate drops off rapidly once the cells accumulate within the activated carbon. Operating with the high level of activated carbon specified in the present application restricts the flow even further. Thus, the fermentation broths discussed by Laustsen cannot be processed using the level of

activated carbon specified by the present invention, even if the fermentation broth is diluted by ~55% as specified by Laustsen. If the broth is diluted to 10 times its original volume (a dilution rate specified by present invention), permeation is more rapid for a while, until the cells collect on the filter cloth, after which the permeation slows dramatically again.

(3) If a cell-free enzyme solution is used according to the claimed invention, the resulting diluted enzyme is readily permeable through the column, whether with the original column frit or with the filter cloth. If the filter cloth is used, the diluted enzyme passes through the column in a matter of seconds. This shows the need for a nearly cell-free solution and the need for a significantly diluted enzyme in order to process through the amount of activated carbon specified by claimed invention.

Also provided in the Rule 132 Declarations are the test results of a large scale production run using several hundred litres of a diluted enzyme solution produced according to the present invention. This work, conducted in 2003/04 in a 20 million gallon per year ethanol plant, demonstrates that the claimed modified alpha amylases surprisingly matched the performance of the industry standard alpha amylase (Liquozyme, from Novozymes) when added to the liquefaction stage of the ethanol plant. These experiments were based on an 80%/20% blend of the presently claimed modified enzymes with Liquozyme. The resulting sugar profiles, fermentation profiles, and flow data showed that the resulting blend was bioequivalent to the 100% Liquozyme, **in spite of the fact that the blend contained 75% less raw alpha amylase**. Clearly, the claimed process was instrumental in dramatically increasing the activity of the alpha amylase. Based on the specified addition rate of 65 mL/min, a 75% reduction in alpha amylase translates into a volumetric savings of around 2100 Litres per month, and thousands of dollars in savings to the ethanol plant.

The Rule 132 Declarations also provide a comparison between the mere dilution of a purified commercial enzyme compared to a diluted purified commercial enzyme that has been processed according to the claimed invention to provide enhanced enzyme activity. The experimental results conclusively demonstrate that the diluted

enzyme processed according the present invention exhibited a surprisingly far greater activity than the merely diluted enzyme.

The experimental evidence of record, both in the originally filed application and the Rule 132 Declarations of record, fully rebut any prima facie case of obviousness raised by the Examiner. Accordingly, withdrawal of all of the Section 103 rejections of record are respectfully requested.

The prior art rejections of record are traversed for the reasons of record and for the following reasons. Applicants have addressed below the new arguments raised by the Examiner in the pending Office Action.

On page 12 of the Office Action, the Examiner argues that:

> Applicant argues that the Declaration submitted demonstrates that the claimed invention results in a surprising 200-900% increase in enzyme activity. Applicants declaration does not appear to be commensurate in scope with the claimed invention or what is disclosed in applicants specification. Applicant is comparing the enzymes, Liquozyme and Allzyme, and their effects on ethanol production and glucose conversion. Applicants third comparison is similar to the second, however, comparing the effects of diluting glucoamylase and its effects on maltodextrin conversion. These comparisons are not commensurate in scope with the claimed invention and introduce confusion as to what "activity" is actually being increased. Is the actual measured activity measured in U/mL or is it the enzymes ability to convert starches and how are those activities intrinsic?

The 26 April 2007 Rule 132 Declaration showed that the % Brix/mg of alpha-amylase enzyme was higher for enzyme processed according to the present invention relative to the % Brix/mg of unprocessed alpha-amylase enzyme. Since % Brix is a known and accepted measurement of sugar in a solution, and the alpha-amylase enzyme produces sugar in it's reaction with starch, a measurement of % Brix/mg of enzyme is a reasonable measurement of activity. While enzyme activity is often referred to in terms of the amount of enzyme required to produce a certain amount of product, enzyme activity can also be referred to in terms of the amount of product produced per amount of enzyme. In our case, since we are concerned with the activity of the processed enzyme *relative* to the raw enzyme, the '/mg' denominator is not needed. Using this relative activity measurement, the measure of sugar need not be

restricted to Brix. Applicants are not bound to use any specific tests and are free to compare the activity of alpha-amylase enzyme, processed according to the method in the present invention, relative to the activity of unprocessed enzyme using any acceptable measurement of product (in this case sugars) in a solution. The 13 March 2008 Rule 132 Declaration confirms this increase in relative activity using % weight, as measured on an HPLC system. Perhaps a better definition of activity is **relative activity,** i.e. the amount of product that results from contacting a certain volume of processed enzyme with substrate at 80 degrees C over 20 minutes relative to the amount of product that results from contacting the same volume of unprocessed enzyme with the same substrate at 80 degrees C over 20 minutes.

Using relative activity as the definition of activity then makes it easy to conclude that alpha-amylase processed according to the current invention is 900% more active relative to unprocessed alpha-amylase (as measured by the amount of product produced per mg of each alpha-amylase). In the 13 March 2008 Rule 132 Declaration, the amount of product (in this case dextrose) produced per milligram of alpha-amylase, processed according to the present invention, is 14 times greater relative to the amount of product produced per milligram of unprocessed alpha-amylase.

The Examiner also argues on page 12 of the Office Action that:

> Laustsen clearly teaches a filtration step to remove cells. Further it is well known in the art that cells and debris clog filters/membranes and interfere with enzyme activity. Therefore such removal of cells is well within the purview of one of ordinary skill in the art when processing a fermentation broth to obtain a desired protein, i.e. enzyme. Applicant states that Laustsens broth cannot be processed according to the applicants invention, but it does not appear that applicant actually carried out the claimed method using Laustsen's broth. Applicant did not remove cells from the broth when processing according to their claimed invention.

Applicants submit that Laustsen's example 2 does not teach filtration of cells prior to contact with activated carbon. Laustsen's example 2 reads as follows:

> A volume of 150 kg Termamyl broth, fermented as described in GB 1,296,839, was diluted to 310 liter with water and 0.300 kg of carbon Picatif FGV 120 together with 6.9 kg of a 45% (w/w) solution of Na.sub.2 Al.sub.2 O.sub.4 from Nordisk Aluminat. pH was adjusted to 10.6, and the

> microfiltration was done in a continuous mode at 45.degree. C. and 60.degree. C. The solutions were microfiltered on a 1 m2 PallSep PS 10 VMF module (0.45 .mu.m PTFE) and at a TMP (transmembrane pressure) equal or below 0.4 Bar. Average permeabilities were calculated as explained below:
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> To obtain a better comparison between the continuous filtration experiments by eliminating any minor differences in TMP, the average permeability has been calculated as follows: Permeability=Flux/TMP (Flux=permeability.times.TMP). The permeability is a measurement of the amount of fouling, e.g. the higher the permeability the smaller the amount of fouling the better pre-treatment method or filtration process.
>
> Process Permeability Trial temperature L/(m.sup.2 * hr * bar) 1 45.degree. C. 138 2 60.degree. C. 157

None of the examples in Laustsen teach a filtration step to remove cells prior to contact with activated carbon. The results in the 13 March 2008 Rule 132 Declaration describe our observations when we replicated Example 2, without applying pressure according to Laustsen, since the claimed invention does not require pressure. Without applying pressure, we could not get the results obtained by Laustsen. This leads us to believe that one skilled in the art would not anticipate that they could follow Example 2 and get an increase in microfiltration process capacity, as is the aim of Laustsen's invention. Nor could one skilled in the art anticipate an increase in specific activity by following this example.

The Examiner states on page 13 of the Office Action that the "applicant's distinct objectives, operating conditions, dilution levels and carbon loading are all quite interesting, pertinent and unexpected," however, the examiner goes on to state that claim 31 does not possess such distinct and necessary limitations.

Claim 31 recites the following limitations:

> (a) diluting an enzyme solution comprising glucoamylase with an aqueous solution by a factor of at least three to provide a diluted enzyme solution;

(b) if the enzyme solution contains cells, filtering the diluted enzyme solution to remove the cells;

(c) treating the diluted enzyme solution with activated carbon at an effective raw enzyme weight to activated carbon weight ratio of not greater than 50:1 and for a sufficient period of time to effect said enhancement; and

(d) removing the activated carbon to provide an enzyme solution of enhanced activity.

The experimental evidence of record in the originally filed application and Rule 132 Declarations is commensurate in scope with claim 31, since these limitations were followed. The dilution and carbon loading requirements are recited in claim 31.

On pages 13-14 of the Office Action the Examiner indicates that Applicants have failed to show evidence commensurate in scope with the present application.

> Specifically, applicant has done a comparison, in Fig. 1 and 2 of a native enzyme solution, the enzyme solution processed through activated carbon and that of amylase produced by the method claimed, i.e. dilution and processing through an activated carbon column. It is unclear how one could conclude that such a graph and comparison would provide unexpected results commensurate in scope with applicant's invention. Applicant also does not provide how such activity is being measured, i.e. specific activity measured in the units as known in Enzymology. Applicant should do a side-by-side comparison of the diluted enzyme without being processed with activated carbon. There is something to be said of an enzyme solution which has been diluted and it's endogenous inhibitors affecting an increase after dilution, i.e. an inhibitor which possesses a low affinity for the enzyme. Changes in activity or inhibition with dilution are a function of the specific enzyme and amount of enzyme in the initial enzyme solution. Further, activated carbon is a known absorbent, therefore the effect may be explained by the fact that the small molecules present in solution are inhibitors of the enzymes therefore binding to the activated carbon, allowing a more pure enzyme to remain. The examiner's position is that a more effective comparison may include additionally the same diluted solution not processed with activated carbon compared to the same volume, amount of enzyme diluted solution which has been processed with activated carbon.

In the 13 March 2008 Rule 132 Declaration, on page 13, an experiment is described in which the Applicants conduct what the Examiner suggested in a prior Office Action. The chart on page 14 shows unequivocally that the diluted glucoamylase

enzyme does not break down the substrate maltodextrin into the products, glucose, as well as the same volume of diluted solution that has been processed with activated carbon according to the present invention.

The 13 March 2008 Rule 132 Declaration shows additional examples to satisfy the Examiner's previous requests. Example 19, on pages 14 – 18 show similar comparisons for fermentation studies. Specifically, Figure 6 shows a comparison of fermentation products for various enzyme formulations. Of note is the glucose production (white bar with horizontal black lines) in a sample of Glucoamylase that has been diluted (1 part glucoamylase and 4 parts buffer), labeled "1:5 Dil GZyme" and processed enzyme (1 part glucoamylase and 4 parts buffer, followed by processing through activated carbon), labeled "Processed Enzyme". Clearly the processed enzyme produces more glucose, which is the product of the enzymatic attack on maltodextrin, than the diluted, unprocessed enzyme.

On page 14 of the Office Action, the Examiner states:

> In response to applicants arguments regarding "intrinsic activity versus relative activity," relative does not appear to be the same as "intrinsic". An intrinsic activity is something which is inherent to the enzyme.

Examples 1, 2 and 3 show that the intrinsic activity of the amylase enzyme is increased upon subjecting the enzyme to the present invention. In the case of Example 1, raw enzyme contained 2035 U/mL of enzyme activity whereas the enzyme subjected to the present invention contained 11000 U/mL of enzyme activity. Both the raw enzyme and the enzyme treated according to the method of the present invention act on starch at the activities outlined above. Therefore, relative to the enzyme from which the treated enzyme was produced, activity has increased. In addition, the amount of starch hydrolyzed as a function of the amount of raw enzyme in solution, the intrinsic activity, has also increased.

On page 14 of the Office Action, the Examiner argues that Laustsen discloses an enzyme dilution of at least a two part dilution, see example 2, which teaches at least 2 parts.

Laustsen's example 2 reads as follows:

> A volume of 150 kg Termamyl broth, fermented as described in GB 1,296,839, was diluted to 310 liter with water and 0.300 kg of carbon Picatif FGV 120 together with 6.9 kg of a 45% (w/w) solution of Na.sub.2 Al.sub.2 O.sub.4 from Nordisk Aluminat. pH was adjusted to 10.6, and the microfiltration was done in a continuous mode at 45.degree. C. and 60.degree. C. The solutions were microfiltered on a 1 m2 PallSep PS 10 VMF module (0.45 .mu.m PTFE) and at a TMP (transmembrane pressure) equal or below 0.4 Bar. Average permeabilities were calculated as explained below:
>
> To obtain a better comparison between the continuous filtration experiments by eliminating any minor differences in TMP, the average permeability has been calculated as follows: Permeability=Flux/TMP (Flux=permeability.times.TMP). The permeability is a measurement of the amount of fouling, e.g. the higher the permeability the smaller the amount of fouling the better pre-treatment method or filtration process.
>
> Process Permeability Trial temperature L/(m.sup.2 * hr * bar) 1 45.degree. C. 138 2 60.degree. C. 157

In the first line, Laustsen specifies that 150 kg Termamyl broth was diluted ***to*** 310 liters with water. Diluting 150L ***to*** 310 L requires that one add 160 L of water. This is a dilution of 1:1.07, not 1:2 as stated by the Examiner.

The Examiner also states on page 14 of the Office Action:

> Applicant also does not show in the submitted Declaration that a dilution of 2:1 is better than or has an unexpected increase of activity compared to a 1:1 dilution.

Applicants have demonstrated this. The Declaration compared the claimed invention to Example 2 of Laustsen, which is a 1:1 dilution using Laustsen's method. Applicants are not required to compare his invention to their own method at a 1:1 dilution.

The Examiner further indicates, on page 14 of the Office Action, that "Applicant does not specifically claim whether or not the cells are present and/or if they have been filtered out. Thus, applicants arguments are not commensurate in scope with the claimed invention." Claim 31 specifically states "if the enzyme solution contains cells,

filtering the diluted enzyme solution to remove the cells;". Therefore, Applicants arguments are commensurate in scope with the claimed invention.

The Examiner argues on pages 14-15 of the Office Action that:

> Applicant argues that the Declaration submitted on 5/2/2007 teaches that filtering an enzyme solution through an activated carbon column does not inherently increase enzyme activity. However, as stated above, the showing in the declaration does not accurately show or compare 'dilution, removal of cells if present, and then contacting with the activated carbon' which results in a surprising enhancement of activity. Applicant merely compares the native enzyme solution, the solution with activated carbon and that which has been diluted. Therefore the arguments are not persuasive.

In the 13 March, 2008 Rule 132 Declaration, Applicants compared the claimed invention, a 10 fold (1,000%) diluted enzyme solution (which is free of cells) processed with an amount of activated carbon to provide enhanced activity, to the cited prior art teaching (1) undiluted enzyme and (2) undiluted enzyme solution merely contacted with activated carbon. Furthermore, in the same Rule 132 Declaration, Applicants have compared the claimed method (dilution of purified enzyme processed with amount of activated carbon to effect enhanced enzyme activity) with a merely diluted enzyme, which conclusively confirms that mere dilution does not result in enhanced enzyme activity. The 13 March, 2008 Rule 132 Declaration demonstrates that the claimed invention provided a surprisingly far greater enzyme activity compared to the enzyme activity of the merely diluted enzyme. Applicants request, again, that the Examiner consider the experimental evidence of record and withdraw the prior art rejections of record.

On page 15 of the Office Action, the Examiner argues that:

> It is noted that applicant has submitted many documents teaching away from applicants claimed invention, i.e. attempting to overcome the inherency rejection and to show unexpected results.
>
> Regarding applicant's arguments directed towards the Shenoy reference, i.e. that Shenoy does not teach purification resulting in activity three times higher than the original non-purified glucoamylase, rather compared to a parent strain. While this has been considered, applicant does not specifically claim, nor show in the Declaration, purification of an original non-purified enzyme. Applicant actually compares in the declaration dated 5/2/2007, an already purified

commercial enzyme to a diluted enzyme purified by the claimed method. Thus, applicant's arguments are not commensurate in scope with the present invention.

As stated in Applicant's response of March 2008, the Applicant's invention requires purification, i.e. removal of cells, only if cells are present before application of the activated carbon. Thus, if an already purified enzyme solution is utilized in the claimed invention, then the step of removing cells is not required since they are already removed. In either case, a purified enzyme solution free of cells is enhanced according to the claimed steps. An already purified enzyme can absolutely be used to practice the claimed invention.

The novelty of Applicants' invention is that the enzyme activity can be greatly enhanced, surprisingly greater than 200%, by the claimed steps of diluting an enzyme solution (which has been purified by removing cells) with an aqueous solution by at least 3 times, and then processing with an amount of activated carbon and for a time sufficient to effect an enhancement of enzyme activity. As correctly pointed out by the Examiner, the examples used purified enzyme, i.e. no cells present. The step of removing cells if present does not alter this experimental evidence, since the cells are absent either way. The enzyme activity of the purified enzyme was conducted according to the claimed steps and, thus, the experimental evidence is commensurate in scope with the claimed invention and must be considered.

The cited prior art does not teach or suggest the claimed steps. Furthermore, the prior art does not teach or even suggest the surprising 200% to 1,000% increase in enzyme activity.

Applicants respectfully submit that the Examiner improperly cites the teachings of Shenoy by assuming that the 3-fold increase in activity is due to purification. Shenoy does not explicitly state that the enhancement of activity is due to purification. Based on the general knowledge in the art, as represented by the numerous references now of record, purification mostly results in reduced enzyme activity and at best the same enzyme activity. Thus there is no support in art or Shenoy for the Examiner's position that Shenoy teaches enhanced activity can be obtained by purification. **While the**

wording in Shenoy is vague, one of ordinary skill in the art would properly interpret the meaning of Shenoy's statements that the enzyme activity enhancement is due to genetic modification in the organism rather than due to purification.

In the second part of the Examiner's argument, reference is made to the May 2, 2007 Rule 132 Declaration, wherein "purification" of an already purified enzyme is discussed. To be clear, the Declaration shows activities of the enzymes before and after they have been processed using the presently claimed technology. The activity of the untreated enzyme, which is a commercially available purified enzyme, has been compared to the activity of this same enzyme after it has been subjected to the claimed process. Since the commercial enzyme has been purified before being subjected to the claimed process, one of ordinary skill in the art would conclude that further purification would have little effect on the enzyme. However, this is not the case. Evidence in the May 2, 2007 Rule 132 Declaration shows that decreasing the enzyme concentration to $1/10^{th}$ the concentration of the original commercial enzyme and subjecting the enzyme to the presently claimed process increases the amount of product relative to the mass of enzyme used. That Applicants can accomplish this dramatic increase in enzyme activity on an already purified enzyme further supports the claimed invention. Simple removal of inhibitors and other "non-essential" proteins would have already been largely accomplished during the manufacturer's purification step, which rules out the Examiner's other hypothesis that enhancement is due to removal of these inhibitors. To see a further 10-fold improvement in activity after Applicants have processed and already purified enzyme is therefore unexpected and fully rebuts the prior art rejections of record.

The Examiner, on pages 15-16 of the Office Action, argues that Applicant's arguments directed to spectral change continue to be confusing:

> Applicant is arguing that it is the goal of purification to avoid a structural and spectral change, yet claim a spectral change, which is distinct from the "raw enzyme solution", as in claim 11,12,13,30. The argument is contradictory. The examiner is well aware of the fact that CD is used after the fact to determine alteration in the structure. Applicant argues that their method is unique in that it

> leads to a change in protein structure. Applicant argues that their method is unique in that it leads to a change in protein structure, due to catalytic modification of the protein. Shenoy also teach that the catalytic activity of a protein, i.e. enzyme is related to its "active" conformation, i.e. secondary and tertiary structure. They state that the ideal purification would preserve protein structure and avoid changes in spectral properties, yet claim that their method is unique in that it leads to a change in protein structure, due to catalytic modification of the protein. This argument is not understood.

It is true that the goal of purification is to avoid a structural and spectral change, as supported by the many references now of record and discussed fully in Applicants' 2 May 2007 Response.

The Examiner's arguments would be well founded if the Applicant's invention were solely purification. Applicants are arguing that the present invention is not solely purification. Purification alone could not explain the dramatic and unexpected increase in enzymatic activity. Therefore, since the present invention does not result solely in purification, there are some other phenomena occurring. These phenomena can, in part, be explained by a shift in CD and UV spectra.

As stated in Applicants' 17 March 2008 response, in a typical purification process, as understood by those of ordinary skill in the art, the goal is to avoid a change in structure, so that activity is mostly preserved. In most cases, a change in structure will decrease, if not completely destroy, activity. In very few cases can a change in structure increase activity. The claimed process is obviously not a typical change in structure, nor is it a typical purification, because an embodiment of the claimed process appears to create a structure that has a higher activity than the original (unprocessed) enzyme. This is why Applicants refer to catalytic *modification* of the protein, without being bound by any theory. Thus, Applicants are reciting in certain claims that the change in structure, as evidenced by the change in CD/UV spectrum, is evidence of the efficacy of the claimed process, because this structural change is associated with an increase in activity, unlike in a conventional purification, where a change in structure leads to a decrease in activity.

On page 16 of the Office Action, the Examiner argues that:

> Applicant argues that the art does not teach or suggest the claimed process of contacting a diluted enzyme solution to activated carbon, in which the cells have been removed. Laustsen by themselves do teach such a process.

The present invention claims the specific conditions required to create the desired effect of the invention. Laustsen does not claim dilution by a factor of at least three, nor does Laustsen claim treating the diluted enzyme solution with activated carbon at an effective raw enzyme weight to activated carbon weight ratio of not greater than 50:1 and for a sufficient period of time to effect an enhancement in enzyme activity. This is because neither Laustsen, nor anyone else of ordinary skill in the art could conceive that by following the steps outlined in the present invention an increase in activity of more than 200% would be observed.

If Laustsen did teach the claimed method, Laustsen surely would have disclosed those method steps and claimed them because the claimed invention surprisingly results in a large reduction in the amount of required enzymes and corresponding reduction in costs associated with purchasing and producing enzymes for use in, but not limited to, renewable fuels production.

On page 16 of the Office Action, the Examiner argues that:

> In response to applicants previous argument dated 10/19/2006 p. 11 of the response, applicant had argued that they do not claim a specific CD spectra, but rather that there had been a change in structure as supported by CD spectra. The examiner's argument, "Applicant absolutely claims a specific CD spectra in claims 12-15, thus applicants arguments are not supported by the claims". Applicant now argues in the response filed 5/2/2007 that this statement is not understood and that claims 11-15 show changes in the defined spectra in the claims. Therefore, in claims 12, applicant does claim a specific CD spectral range.

Respectfully, if Applicants claimed a specific CD spectra the claim would look something like this:

> A method as defined in claim 1 wherein said enzyme solution of enhanced activity shows an ellipticity per residue of between -35×10^{-3} and -45×10^{3} at 206 nm
>
> Or

> A method as defined in claim 1 wherein said enzyme solution of enhanced activity shows a Molar Ellipticity of -110 at 300nm

Also, one of ordinary skill in the art would not claim a "spectral range" as the "spectral range" is the independent variable. The spectral range is the range of wavelengths of light at which ellipticity is measured, therefore claims would likely be made on either a combination of dependent and independent variables or on the dependent variable which is molar ellipticity or ellipticity per residue rather than only the spectral range itself.

Reading claims 11 to 15 therefore indicates to one skilled in the art that the enzyme that has been subjected to the present invention has "visible spectra distinct from said raw enzyme solution" and "relative absorbance intensity lower than said raw enzyme solution in the spectral range of 205 – 230 nm". Applicants are not claiming a spectral range of 205 – 230 nm. Applicants are simply claiming that in the 205 – 230 nm range, the molar ellipticity or relative absorbance intensity of the treated enzyme is lower than the molar ellipticity or relative absorbance intensity of the raw enzyme in the same 205 – 230 nm spectral range.

It is impossible for Applicants to claim a specific spectra because the spectra of the enzyme subjected to the present invention is *dependent* on the spectra of the raw enzyme. Applicants are claiming that between 205 and 230 nm, the processed enzyme will have a lower relative absorbance intensity than the raw enzyme.

Each of claims 11-15 indicate changes in spectra relative to the unprocessed raw enzyme.

On pages 16-17 of the Office Action the Examiner argues that:

> Applicant argues that one of skill in the art would not have been motivated to dilute an enzyme prior to purification with activated carbon because the art does not suggest so. However, Laustsen does teach dilution prior to purification. In response to applicant's arguments, that there is no motivation or teaching/suggestion, applicant is advised that KSR forecloses the argument that a specific teaching, suggestion or motivation is required to support a finding of obviousness. See the recent board decision Ex parte Smith, --USPQ2d--, slip op at 20,(Bd. Pat.App Y Interf. June 25, 2007)(citing KSR,82 USPQ2d at 1396) (available at http://www.uspto.govt/web.offices/dcom/bpai/prec/fd071925.pdf

Applicants did not only argue "that one of skill in the art would not have been motivated to dilute an enzyme prior to purification with activated carbon because the art does not suggest so" as alleged by the Examiner. Applicants also pointed out how mere dilution of Laustsen's method still does **not** result in the claimed invention. The Rule 132 Declaration submitted on 17 March, 2008 comparing the claimed invention to the specific teachings in Laustsen confirms such.

Furthermore, this Rule 132 Declaration and the previous Rule 132 Declaration of record confirm the unexpected advantages of the claimed invention compared to the prior art. Applicants are not required to compare their invention to a theoretical method that does not exist. Applicants are only required to compare their invention to what is known in the art, i.e. actual teachings, such as an experimental example. KSR specifically states that unexpected advantages overcome a prima facie case of obviousness. Thus, according to the holding in KSR, the Section 103 rejections should be withdrawn in view of the unexpected results confirmed by the experimental evidence of record in the originally filed application and Rule 132 Declarations of record.

On page 17 of the Office Action, the Examiner argues that:

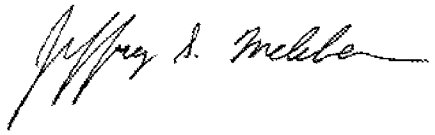
In response to the applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are base on combination of references. See *In re Keller*, 642 F.2d 413,208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

Applicants have not attacked the references individually. In addressing the rejection based on the combination, Applicants have clearly pointed out what each reference in fact teaches (and fails to teach) and then shows how the combination of those teachings fails to make obvious the claimed invention.

In view of the many differences between the claimed invention and the prior art and theoretical combination of references, and the unexpected advantages of the claimed invention, withdrawal of the Section 103 rejection is respectfully requested.

In view of all of the objections and rejections of record having been addressed, Applicants submit that the present application is in condition for allowance and Notice to that effect is respectfully, requested.

Respectfully submitted,
Manelli Denison & Selter, PLLC



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